UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS GRADUAÇÃO EM MEDICINA VETERINÁRIA

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CONCORDÂNCIA ENTRE MÉTODOS DE DIAGNÓSTICO PARA BACTERIÚRIA EM CÃES

Santa Maria, RS

2023

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Medicina Veterinária, Área de concentração em Patologia e Patologia Clínica Veterinária, da Universidade Federal de Santa Maria (UFSM, RS), como requisito para obtenção do título de **Mestre em Medicina Veterinária**

Orientadora: Professora Drª. Cinthia Melazzo de Andrade

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Aprovado em 10 de fevereiro de 2023.

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AGRADECIMENTOS

Aos meus pais e a minha irmã por me apoiarem imensamente nessa árdua jornada. Obrigada imensamente por me estimularem a continuar estudando em um país que sofreu tantos ataques a Educação e a Ciência nos últimos quatro anos.

À minha professora e orientadora Cinthia por ter me recebido e me dado a oportunidade de realizar mestrado na UFSM. Sou grata pelos ensinamentos, que com certeza contribuirão para a minha experiência profissional. Ao professor Alexandre Krause, pela paciência e dedicação ao ensinar todos os membros do laboratório.

Agradeço, com todo o meu coração, ao LCV (antigo LACVET) por me receberem tão bem. Nunca tinha me sentindo tão acolhida em pouquíssimo tempo. Certamente, vocês transformaram o ambiente laboratorial em um segundo lar. A capacidade de ajudar ao próximo vai ser algo que levarei para sempre em minha vida.

Gostaria de agradecer aos meus colegas e amigos de laboratório. Bernardo, meu pai gaúcho. Camila, por tirar minhas dúvidas de citologia e ser uma referência como profissional. Andreza e Lara por me ajudarem quando eu mais estava desesperada com trabalhos acadêmicos; Jamile, Renata, Nathalia, por escutarem minhas angústias. Jenifer, me acompanhar nas dificuldades de ser vegetariano. Vini, obrigada por todas as caronas para o RU, companhia nas festas em Santa Maria e por eu poder contar sempre contigo quando preciso, já tem um espaço reservado no meu coração. As R2 do LCV, Bruna e Anne, por terem me auxiliado no meu projeto e por serem lindas, maravilhosas e me aguentar todas as tardes. Obrigada Dani, seu Wilson, Rose por trazerem alegria e bom humor para o laboratório.

Ao Bruno Almeida e Larissa, que por mais que trabalhemos mais juntos, está sempre disponível para me auxiliar quando eu preciso.

À Rose e Belle, por escutarem quando eu estou triste e me motivar a seguir na Medicina Veterinária, um caminho repleto de obstáculos.

Ao Programa de Pós-Graduação em Medicina Veterinária (PPGMV-UFSM) e à CAPES.

Obrigada aos melhores seres vivos do mundo e que aprendo a lidar com a falta deles: Scotch, o melhor cão que já existiu, Berilão, te encontrar com certeza foi um sinal para eu escolher Veterinária, Pussycat, meu primeiro amor felino de Santa Maria, Berilinho Rabo Reto, Minie, Grafite e Filó.

RESUMO

CONCORDÂNCIA ENTRE MÉTODOS DE DIAGNÓSTICO PARA BACTERIÚRIA EM CÃES

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Em medicina veterinária, o diagnóstico de bacteriúria comumente baseia-se somente na avaliação do sedimento urinário não corado, este método não é o mais adequado, pois partículas amorfas presentes na urina podem ser confundidas com bactérias. Portanto as amostras de urina dos pacientes com suspeita de infecção do trato urinário devem ser enviadas para a realização de cultura bacteriológica, considerado "padrão ouro" de diagnóstico. No entanto, devido ao maior tempo exigido para a obtenção dos resultados, esse procedimento nem sempre é realizado. Nesse contexto, a realização da análise do sedimento urinário corado se apresenta como um método potencialmente mais preciso que a avaliação do sedimento não corado. Assim, este estudo tem como objetivo investigar se a análise de sedimentos urinários corados é um método de triagem eficiente, para identificação de bacteriúria quando comparado à cultura microbiológica. As amostras de urina utilizadas foram provenientes das amostras de rotina do Laboratório Clínico Veterinário (LCV) localizado no Hospital Veterinário Universitário (HVU) da Universidade Federal de Santa Maria (UFSM). Naurinálise, foram realizadas as análises físicas, químicas e análise do sedimento não corado e corado com Gram, sendo incluídas neste estudo amostras coletadas pelo método de micção espontânea e cateterismo. Houve exclusão de amostras com menos de 5 ml e de pacientes em tratamento com antibióticos. Foram destinados 10ml de urina para urinálise, enquanto 1ml de urina foi destinada à realização de cultura bacteriológica quantitativa no Laboratório de Bacteriologia (LABAC) da UFSM. Para a técnica de colheita de urina por cateterismo, 55% (n=40) dos resultados foram detectados como falso positivos para análise do Sedimento Urinário (SU) não corado e 2,5% (n=40) dos resultados foram avaliados como falso negativos para análise do SU corado. Por fim, para colheita de urina por micção espontânea, 23,3% (n=60) dos resultados foram detectados como falso positivo na análise do SU não corado e 1,66% (n=60) dos resultados observados no SU foram detectados como falso negativos. A técnica de coloração de Gram do SU aumentou sensivelmente a diferenciação entre detecção de substâncias amorfas e bacteriúria nas amostras de urina de cães, sugerindo a aplicabilidade desta coloração na rotina laboratorial.

Palavras-chave: Urinálise. Sedimento urinário corado. Cultura bacteriana. Sensibilidade. Bacteriúria. Gram

ABSTRACT

AGREEMENT BETWEEN DIAGNOSTIC METHODS FOR BACTERIURIA IN DOGS

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In veterinary medicine, the diagnosis of bacteriuria is commonly based only on the evaluation of unstained urinary sediment, this method is not the most appropriate, as amorphous particles present in the urine can be confused with bacteria. Therefore, the urine sample of patients with suspected urinary tract infection should be sent for bacteriological culture, which is considered the "gold standard" for diagnosis. However, due to the longer time required to obtain results, this procedure is not always performed. In this context, carrying out the analysis of stained urinary sediment is a potentially more accurate method than the assessment of non-stained sediment. Thus, this study aims to investigate whether the analysis of stained urinary sediments is an efficient screening method for the identification of bacteriuria when compared to microbiological culture. The urine samples used came from the routine samples of the Laboratory of Veterinary Clinical Analysis (LCV) located at the University Veterinary Hospital (HVU) of the Federal University of Santa Maria (UFSM) after the urinalysis in which the physical analyses, chemical analysis and analysis of slides of unstained and Gram-stained urinary sediment, included in this study, collected by the urination method with the eism catheter. There was exclusion of Exception with less than 5 ml and patients under treatment with antibiotics. 10ml of urine was used for urinalysis, while 1ml of urine was used for quantitative bacteriological culture at the Bacteriology Laboratory (LABAC) at UFSM. For the technique of urine collection by catheterization, 55% (n=40) of the results were detected as false positives for analysis of unstained SU (Urinary Sediment) and 2.5% (n=40) of the results were evaluated as false negative for stained SU analysis. Finally, for spontaneous micturition urine collection, 23.3% (n=60) of the results were detected as false positives in the analysis of the US not detected and 1.66% (n=60) of the results observed in the SU were detected as a false negative. The SU Gram color technique significantly increased the differentiation between detection of amorphous substances and bacteriuria in dog urine samples, suggesting the applicability of this color in laboratory routine.

Keywords: Urinalysis. Stained urine sediment. Bacterial culture. Sensitivity. Bacteriuria. Gram

LISTA DE TABELAS

SUMÁRIO

1 INTRODUÇÃO

A infecção do trato urinário (ITU) é definida como adesão, multiplicação e persistência de um agente infeccioso no sistema urogenital, que geralmente se constitui em uma bactéria (BARTGES, 2004), geralmente, provenientes do intestino ou da pele que circunda o períneo (SMEE et al., 2013). A ITU canina é uma enfermidade comum na rotina clínica, ocorrendo em aproximadamente 14% dos cães que já passaram por alguma avaliação clínico-veterinária (THOMPSON et al., 2011). É mais comum em fêmeas com idade média de 7-8 anos, mais facilmente descoberta quando os animais apresentam sinais clínicos, como hematúria, polaquiúria e aumento da frequência urinária (YAMAKA et al., 2019). No entanto, a ITU pode existir sem sinais clínicos, e nestes casos o diagnóstico é por acaso (CHEW; DIBARTOLA; SCHENCK, 2011).

O diagnóstico de ITU por meio da urinálise pode direcionar para um exame de sedimento urinário normal, enfatizando a necessidade de um resultado de cultura positivo para confirmar o diagnóstico de ITU (THOMPSON et al., 2011). A identificação de bactérias na urina não é sinônimo de ITU, podendo representar contaminação da amostra, especialmente se a amostra for coletada por micção natural ou cateterismo uretral em que bactérias do trato urogenital distal ou a urina pode ser contaminada no procedimento de coleta (BARTGES, 2004).

Em relação à etiologia, a maioria das ITUs em cães envolve um único agente. A bactéria Escherichia coli é responsável pela maior parte das infecções registradas em cães seguido por cocos gram-positivos, incluindo Proteus, Klebsiella, Pasteurella, Pseudomonas, Corynebacterium, e vários outros gêneros raramente relatados (OLIN & BARTGES, 2015). Existem relatos na literatura veterinária que registram a prevalência de bacteriúria em cães com doença clínica, por exemplo, diabetes melito, hiperadrenocorticismo, doença do disco intervertebral, parvovirose canina e urolitíase (MCGHIE; STAYT; HOOSGOOD, 2018).

A ITU inferior pode ser classificada como cistite bacteriana esporádica (não complicada) ou recorrente (complicada) (WEESE et al., 2011; WEESE et al., 2019). Cães acometidos pela cistite bacteriana esporádica normalmente apresentam polaquiúria, disúria e/ou estrangúria como sinais clínicos, manifestando de um a dois episódios de suspeita de cistite em um período de 12 meses (WEESE et al., 2019). A cistite bacteriana recorrente, por sua vez, caracteriza-se pela ocorrência de três ou mais episódios de cistite clínica em um

prazo de 12 meses ou 2 ou mais episódios em um período de seis meses (WEESE et al., 2019). Apesar de ser denominada como cistite bacteriana recorrente, essa terminologia também inclui reinfecções, podendo ser pelo mesmo ou outro agente (WEESE et al., 2019).

A análise apenas do sedimento urinário é inadequada para o diagnóstico de ITU devido a problemas, como a ocorrência de resultados falso positivo de bacteriúria na ausência de infecção clínica (SWENSON et al., 2004). A cultura bacteriana aeróbica e o teste de suscetibilidade devem ser realizados em todos os casos, para confirmar a presença de infecção, identificar a presença de bactérias resistentes, que podem não responder à terapia inicial, e fornecer ao clínico-veterinário quais são as bactérias mais comuns em sua rotina clínica (WEESE et al., 2011; (GRANT; NAPIER; CORRIGAN, 2021).

As amostras de urinas destinadas a cultura microbiológica devem ser coletadas por cistocentese (CHEW; DIBARTOLA; SCHENCK, 2011), pois este método não necessita passar pela uretra distal, que é habitada por bactérias comensais mesmo em cães saudáveis (GRANT; NAPIER; CORRIGAN, 2021). Após a coleta de urina para cultura bacteriológica, a amostra deve ser analisada dentro de 30 minutos, se isso não for possível, essas amostras devem ser refrigeradas a temperaturas em torno de 4 a 5°C por até seis horas sem alteração drástica nas populações bacterianas (DUNNING & STONEHEWER, 2021). No entanto, alguns organismos urinários mais exigentes podem morrer com períodos de armazenamento prolongados, o que pode levar a uma contagem bacteriana quantitativa mais baixa (DUNNING & STONEHEWER, 2021). A realização da cultura bacteriana é importante para descartar a ocorrência de doenças não infecciosas que mimetizam a ITU, direcionando assim tratamento e promover a administração antimicrobiana corretamente, evitando o uso indiscriminado de antimicrobianos, que contribuem para a resistência bacteriana aos antimicrobianos (GRANT; NAPIER; CORRIGAN, 2021). O custo e o tempo necessários para a cultura bacteriana têm sido apontados como possíveis empecilhos para a realização deste exame na prática clínica (DE BRIYNE et al., 2013;).

O uso histórico de antimicrobianos provocou o desenvolvimento de resistência bacteriana em seres humanos com ITUs, destacando a importância da seleção correta de um antimicrobiano no tratamento de indivíduos com ITU (WONG; EPSTEIN; WESTROPP, 2015). Na Medicina Veterinária, o surgimento de patógenos multirresistentes também torna difícil a realização da terapêutica de pacientes com ITU devido às escolhas limitadas de medicamentos (WEESE et al. 2011). A diminuição da eficácia de fármacos contra bactérias

causadoras de ITU em cães tem sido associada ao uso empírico de antimicrobianos que não deve substituir a cultura na escolha da terapia medicamentosa. O tratamento de infecções causadas por bactérias resistentes é muito difícil, podendo causar morbidade significativa aos animais, além de tornar-se oneroso financeiramente aos tutores (JOHNSTONE, 2019).

O surgimento de bactérias multirresistentes (resistentes a três ou mais categorias de antimicrobianos) em animais de companhia é uma preocupação crescente na medicina veterinária, se constituindo em questão de saúde pública, uma vez que os animais de companhia podem desempenhar um papel na disseminação de bactérias resistentes devido ao seu contato próximo com humanos (MARQUES et al., 2016). A realização da urocultura evita o desenvolvimento de resistência antimicrobiana, reduz o custo geral do tratamento e, mais importante, a morbidade do paciente. Além de aumentar a chance de selecionar o medicamento mais adequado já no início do tratamento (HALL; HOLMES; BAINES, 2014). A administração de antimicrobianos é frequentemente usada empiricamente com base, apenas, na presença de sinais clínicos compatíveis e na urinálise, sem os resultados do exame de urocultura. Geralmente, a terapêutica contra a ITU é implementada para aliviar os sintomas de ITU (MARQUES et al., 2016). Nos casos em que não há possibilidade de realização de cultura bacteriana, a seleção antimicrobiana deve ser baseada em características bacterianas observadas no sedimento urinário. No entanto, existem desvantagens a serem consideradas com a utilização de antibióticos empíricos, por exemplo, a identificação de cocos gram-positivos pode também ser compatível com *Enterococcus* spp., que seria resistente a uma cefalosporina (SMEE et al., 2013). Além disso, partículas amorfas semelhantes a bactérias (pseudobactérias) são comumente detectados na avaliação microscópica do sedimento urinário não coradas, sendo relatadas como bactérias por patologistas clínicos com diferentes tempos de experiência. Foi relatado que o uso da coloração de Gram no sedimento urinário melhorou substancialmente a identificação de bactérias em amostras de urina obtidas de humanos. Similarmente, a coloração do sedimento urinário pelo Wright-Giemsa melhorou significativamente a sensibilidade, especificidade e eficiência do teste de detecção microscópica de bacteriúria em comparação com a do método do sedimento urinário não corado, se constituindo um método rápido, barato e de fácil execução (SWENSON et al., 2011).

Portanto o objetivo desse trabalho foi avaliar os resultados da análise do sedimento urinário não corado e do sedimento corado com Gram em relação ao exame padrão-ouro,

cultura bacteriológica. As amostras de urina utilizadas no presente estudo foram obtidas da rotina de atendimento do Hospital Veterinário Universitário (HVU) da Universidade Federal de Santa Maria com intuito de demonstrar que a utilização da coloração de Gram no sedimento urinário, auxiliaria o patologista clínico na diferenciação de estruturas parecida com bactérias no sedimento urinário, evitando a prescrição de antibióticos de forma desnecessária, já que, muitas vezes, o tutor não dispõem de recursos financeiros para pagar a cultura bacteriológica ou não há a possibilidade de esperar pelos resultados desse exame.

2 MANUSCRITO

Os resultados deste trabalho encontram-se na forma de manuscrito, o qual será submetido para a revista Ciência Rural. As normas da revista podem ser conferidas no site: http://coral.ufsm.br/ccr/cienciarural/normas.htm

Agreement between diagnostic methods for bacteriuria in dogs

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ABSTRACT

Diagnosis of bacteriuria in veterinary medicine is commonly based on unstained urinary sediment (US) evaluation. Nonetheless amorphous particles can be confused with bacteria. This study aims to investigate whether the stained sediment increases the sensitivity of bacteriuria detection. Forty-seven 7ml urine samples from dogs of different breeds, sex, age and collection method were analyzed, destined for the Veterinary Clinical Analysis Laboratory (LCV) located at the University Veterinary Hospital (HVU) of the Federal University of Santa Maria (UFSM). In addition, an aliquot of 2 ml of these samples was destined for quantitative bacteriological culture, in the bacteriology laboratory (LABAC) of UFSM to compare the results obtained in the analysis of the US, because it is the gold standard, for the diagnosis of bacteriuria. For the technique of urine collection by catheterization, 55% (n=40) of the results were detected as false positives for analysis of unstained SU (Urinary Sediment) and 2.5% (n=40) of the results were evaluated as false negative for stained SU analysis. Finally, for spontaneous micturition urine collection, 23.3% (n=60) of the results were detected as false positives in the analysis of the US not detected

and 1.66% (n=60) of the results observed in the SU were detected as a false negative. Performing GRAM staining of urinary sediment increased the differentiation between amorphous substances and bacteriuria in urine samples from dogs, which suggests the applicability of this staining in laboratory routine.

Key words: Urinalysis, stained urine sediment, bacterial culture, sensitivity, bacteriuria, Gram.

INTRODUCTION

Urinary tract infection (UTI) is a common disease in the clinical routine of small animals, occurring in approximately 14% of dogs that undergo clinical-veterinary evaluation. (BARTGES, 2004). Bacterial culture is necessary to confirm the diagnosis of UTI (THOMPSON et al., 2011), as urinalysis may erroneously lead to an unaltered exam. Cystocentesis is the most recommended collection method for samples that will be destined for culture, as this method avoids contact with the distal urethra, which is inhabited by bacteria, even in healthy dogs (GRANT et al., 2021). Therefore, urine samples may present contamination, especially if the sample is collected through natural urination or urethral catheterization (BARTGES, 2004). Opposite to recommendations, many veterinarians send urine samples by spontaneous urination for culture, even in patients who show signs consistent with UTI (Urinary Tract Infections), as it is a simple and non-invasive procedure that can be performed by the tutors themselves (GRANT et al., 2021).

Bacterial culture and susceptibility testing confirms infection, allows identification of resistant bacteria, and rules out non-infectious diseases that mimic UTI (WEESE et al., 2011). This approach avoids the indiscriminate use of antimicrobials, which would increase bacterial resistance to these drugs (GRANT et al., 2021). The advantage of performing a culture lies in

the possibility of determining the level of bacterial growth, by counting colony forming units (CFUs), which can be used in the interpretation of results (WEESE et al.,2011).

However, the cost and time required for bacterial culture have been identified as possible obstacles to performing this test in clinical practice (DE BRIYNE et al., 2013). Thus, in some cases, the administration of antimicrobials is performed based on clinical signs and urinalysis findings compatible with UTI (MARQUES et al., 2016).

In the microscopic analysis of urinary sediment, amorphous particles similar to bacteria (pseudobacteria) are commonly reported as bacteria, regardless of the experience of the clinical pathologist who performs the examination (SWENSON et al., 2004). In addition, the use of Gram or Wright-Giemsa stains on urinary sediment in dogs and cats substantially improves the identification of bacteria when compared to analysis of unstained urinary sediment (SWENSON et al., 2011), which can minimize the previously raised issue.

Knowing that the possibility of mistakenly identifying bacteria is a reality in routine and that the manufacture of stained slides of urinary sediment is a quick, inexpensive and relatively easy procedure (SWENSON et al., 2011) this study aims to to verify the concordance between the analysis of the urine sediment stained or not, with the bacterial culture of urine of dogs for the confirmation of bacteriúria.

MATERIALS AND METHODS

The study was approved by the Animal Ethics Committee under protocol number 9964140622. Urine samples from female (n=48) and male (n=62) dogs, of different breeds, ages and collection methods, derived from routine Clinical and surgical tests at the University Veterinary Hospital (HVU) at the Federal University of Santa Maria (UFSM) were processed for the detection of bacteriuria by analysis of the sediment stained or not by the Gram method.

A 2 mL aliquot of these samples was sent for bacteriological culture. Samples with less than five ml of urine or urine from animals treated with antibiotics were excluded from the experimental design.

Unstained sediment analysis

Five milliliters of urine were centrifuged at 1500 rpm for 5 minutes. After this process, the volume of 0.5 mL of supernatant was maintained, which was homogenized with the sediment. With the aid of a pipette, 10µL of this mixture was placed on a glass slide and superimposed by a cover slip for microscopy. Ten fields of the sediment were analyzed in an optical microscope (Zeiss®) with a 10X and 40X objective. The average number of bacteria was classified in a 400X objective, being classified as none, occasional (<3 bacteria), few (3 to 10 bacteria), moderate (11 to 40 bacteria) and severe (> 40 bacteria) (SWENSON et al., 2004).

Gram stained sediment analysis

The slides with the urinary sediment were subjected to dry fixation and then stained with a Gram staining kit (Laborclin®) with the methodology according to the manufacturer's instructions. The stained slides were examined in an immersion objective (1000X) in 20 fields, being classified as none, occasional (1 to 4 bacteria), few (5 to 9 bacteria), moderate (10 to 20 bacteria) or severe (> 20 bacteria). (SWENSON et al., 2004).

Aerobic urine culture

A 0.01 mL aliquot of urine was seeded and enriched in Blood and MacConkey Agar medium using a sterile calibrated loop. Blood agar medium was used to promote the growth

of fastidious organisms. There was a serial dilution of the urine plate ranging from 10^{-1} a 10^{-6} and 0.1 ml of urine was inoculated in each dilution on the surface of each agar plate in duplicate.

In collections by spontaneous urination, it was defined as significant bacteriuria from 100,000 CFU/ml. In urine collected by voided specimens, samples with growth < 1000 CFU/ml were considered evidence of contamination (SWENSON et al., 2004).

Statistical analysis

The Kappa coefficient test (95% confidence interval) was performed to calculate the concordance between the results of the culture with the analysis of the stained sediment and the unstained sediment. In addition, sensitivity, specificity, accuracy, positive and negative predictive value were also calculated.

RESULTS AND DISCUSSION

100 urine samples were received during the period from May to July 2022, two samples were excluded from the study due to contamination in the bacteriological culture, with 60 positive samples and 40 negative samples in the culture. 60 samples were collected through voided specimens and 40 through catheterization, 48 from females and 52 from males. Two dogs each provided two urine samples, the rest of the patients only one sample was collected. Quantitative aerobic urine culture was positive in 15 samples collected by catheterization (25%) and in 45 samples collected by voided specimens (75%). In this last collection method, there were 72 bacteria.

Microorganisms isolated by free cacth include: *Staphylococcus* sp. (n=21, 29.16%), *Escherichia coli* (n=15, 20.83%), *Streptococcus* sp. (n=10, 13.88%), *Klebsiella* sp. (n=6, 8.33%), *Acinetobacter* sp. (n=5, 6.94%), *Enterococcus* sp. (n=4, 5.55%), *Pseudopmonas* sp.

(n=4, 5.55%), *Proteus* sp. (n=3, 4.16%), *Micrococcus* sp. (n=2, 2.77%), *Neisseria* sp. (n=1, 1.38%) e *Corynebacterium* sp. (n=1, 1.38%). By catheterization, 18 bacteria were isolated. Microorganisms isolated by were: *Escherichia coli* (n=6, 33.3%); *Staphylococcus* sp. (n=4, 22.2%), *Streptococcus* sp. (n=4, 22.2%); *Proteus* sp. (n=1, 5.55%), *Pseudomonas* sp. (n=1, 5.55%), *Klebsiella* sp. (n=1, 5.55%), *Acinotobacter* (n=1, 5.55%). Bacterial cultures in which only one species was isolated were 31 of 60 (51.66%) and cultures in which multiple, 2 or more species were grown were 29 of 60 (48.33%). The bacteria most found in the present study were *Staphylococcus* sp and *Escherichia coli*, which is in agreement with other authors (WAY et al., 2013; SWENSON et al., 2004). *Escherichia coli* is one of the most isolated bacteria in canine UTI patients followed by Gram positive cocci: *Staphylococcus* sp. *Proteus* sp., *Klebsiella* sp., *Pasteurella* sp., *Pseudomona* sp., *Corynebacterium* sp. (OLIN & BARTGES, 2015; GATORIA et al., 2006).

Regarding the samples collected by catheterization (n=40), bacteriuria was observed in 37 samples by analysis of unstained sediment, in 15 samples by stained sediment and in only 10 by bacterial culture (Table 1). Twenty-two false positive results were observed in the unstained sediment analysis, five false positive results in the stained sediment analysis and four false negative results in the stained sediment analysis (Table 2).

For samples collected by voieded specimens (n=60), bacteriuria was observed in 58 samples in the unstained sediment analysis, 35 in the stained sediment analysis and 15 in the bacterial culture (Table 1). There were 14 false positive results for the unstained sediment analysis, 3 false positive results for the stained sediment, one false negative result for the unstained sediment analysis and one false negative result for the stained sediment analysis (Table 2).

The concordance of the format of bacteria found in the stained and unstained sediment was analyzed with the gold standard method, culture (Table 3). There was the observation of 40 samples without bacterial growth, in samples collected by catheterization and spontaneous urination, in which in the analysis of the unstained sediment, five samples (12.5%) were visualized without bacteria and 35 samples with bacteria of the cocci type (87.5%) (Table 4 and 5).

When the analysis of the stained sediment was performed, no growth was observed in 32 samples (80%) and in eight (20%) samples, cocci bacteria were observed. As for the cultures in which coccoid-shaped bacteria were isolated (n=7), the unstained sediment technique provided six results (85.7%) compatible with cocci and one result compatible with a mixed population, cocci and bacilli (16.7%). Regarding the cultures in which there was growth of bacilli (n=10), it was observed in the analysis of the unstained sediment, four results compatible with cocci (40%) and six compatible with cocci and bacilli (60%). When the stained sediment was analyzed, two samples (20%) were seen without the presence of bacteria and 1 (10%) samples had coccus-type bacteria, 6 had bacilli (60%) and one (10%) mixed population was visualized. As for the bacterial cultures in which mixed populations of bacteria were isolated (n=8), it was observed in the analysis of the unstained sediment, one result with no bacteria (12.5%), five results compatible with cocci (62.5%) and two results (25%) compatible with mixed population. In the analysis of the stained sediment, there were two samples without bacteria (25%), four samples with only cocci-type bacteria (50%), one sample (12.5%) with only bacillary bacteria and one that presented (12.5%) mixed population.

Analysis of the unstained sediment showed a Kappa coefficient of 0.009 (95% CI 0.057 to 0.077) when compared to the culture. The calculated sensitivity was 98.39%, specificity

2.44%, positive predictive value 60.40%, negative predictive value 50.00% and accuracy 60.19%

The identification of bacteriuria in the stained sediment showed a Kappa coefficient of 0.460 (95% CI 0.293 to 0.626) when compared with the culture. The calculated sensitivity was 67.74%, specificity 80.49%, positive predictive value 84.00% and negative predictive value 62.26% and accuracy of 72.82%.

The analysis of the Gram sediment proved lower sensitivity and specificity compared to other studies (WAY et al., 2013, NEIL et al., 2013). This divergence occurred because of the different populations included in the study and because the urine collection methods were not the same, the present study used samples from catheterization and voieded specimens, while the authors mentioned above obtained the samples by cystocentesis.

Despite the high sensitivity of unstained urinary sediment analysis (98.9%), and its low specificity (2.44%), this method is not suitable for use as a screening test for bacteriuria, requiring values of high specificity and sensitivity (GOULART & CHIARI, 2007). Based on this study, 27 (67.5%) of 40 samples without bacterial growth, no bacteria were seen in the analysis of the stained sediment, but when the analysis of the unstained sediment occurred, there was the observation of bacteria which could result in antimicrobial therapy unnecessary.

Analysis of the stained sediment revealed a Kappa coefficient of 0.460 (95% CI 0.293 to 0.626), which indicates a moderate concordance with the culture, while analysis of the unstained sediment evidenced a Kappa coefficient of 0.009 (95% CI 0.057 to 0.077), which constitutes a minimal correlation. Therefore, the Gram urinary sediment has a better concordance with the culture, the gold standard method for detecting bacteriuria, making the analysis of the stained sediment a more reliable test than the observation of the unstained sediment.

In the present study, the Odds ratio, positive and negative, was also calculated, which studies the probability of an event occurring in two different tests. The analysis of the stained sediment showed a higher positive Odds ratio value (3.47) than the unstained sediment (1.01), indicating a greater chance of simultaneous occurrence of positive results from the sediment color method with the gold standard test. This data reinforces what was seen by the Kappa coefficient, a greater association between the analysis of the stained sediment and the culture, reinforcing the reliability of the Gram urinary sediment. This greater credibility was also confirmed by the high accuracy presented by the analysis of stained slides (72.87%) demonstrates that this test is more likely to provide correct results when compared to the analysis of the unstained sediment, which presented an accuracy of 60.19%.

Gram staining of the sediment showed a high positive predictive value, constituting a good indicator of bacteriuria, but a lower negative predictive value, demonstrating that negative results in stained urine slides would be less reliable than positive results. The analysis of the unstained sediment, in other way, presented lower positive and negative predictive values than the analysis of the stained sediment. These lower values are due to the low specificity and high sensitivity obtained by the unstained method, which mainly reduced the negative predictive value of this test. The present study had lower positive and negative predictive values compared to other studies (WAY et al., 2013; SWENSON et al., 2011; NEIL et al., 2013). In the study carried out by WAY et al. (2013) the positive and negative predictive value were 100% and 93%, respectively. In the study carried out by NEIL et al. (2013), a positive and negative predictive value of 88% and 98%, respectively, was found. While in the study executed by SWENSON et al. (2004), there was the observation of positive and negative predictive value, respectively, of 94.5% and 98.7%. This difference may have been caused by differences in the population used in the studies and by the urine

collection method used. Urine samples in the present study were collected by catheterization and voieded speciemens, which are methods more susceptible to contamination by bacteria from the lower urinary tract. Sample contamination can be confirmed by performing a quantitative bacteriological culture obtained by catheterization and voieded speciemens (SMEE et al., 2013). However, it is reliable to use the voieded speciemens method, if veterinary reference intervals are used for counting CFUs/ml, if the samples are refrigerated and cultured within 4 h after collection (SOARESEN et al., 2016). Despite the high prevalence (60.19%) of positive cultures (n=60), most of these cultures showed CFU count values (Colony Forming Units) lower than 10,000 CFUs/ml in the collection by catheterization and below of 100,000 CFU/ml in the collection by voided speciemen, not being sufficient for the detection of bacteriuria. Thirty samples from voided speciemen were observed, representing 66.7% of the cultures with bacterial isolation in this collection method, and 5 by catheterization, totaling 33.3% of the samples with bacterial growth by catheterization.

All incorrectly identified samples were classified as cocci-type bacteria, both in the analysis of the stained sediment and the unstained sediment technique. This occurs because bacilli are easier to differentiate due to their unique shape, resulting in fewer false-positive results (SØRENSEN et al., 2016; MONTEIRO et al., 2021). However, despite the morphology of bacillary bacteria being easier to distinguish, bacterial cultures with a mixed population showed a lower percentage of correct identification of bacilli in the stained and unstained sediment. In the stained sediment, only one sample was identified the presence of cocci and bacilli in the Gram and in two samples in the non-stained sediment. Possibly, the failure in the diagnosis of mixed populations is due to the low presence of CFUs of bacillary or coccoid bacteria, poor preparation of the urinary sediment slide, contamination of the

culture medium and pre-analytical errors (urine storage), which may favor or inhibit bacterial growth (WAY et al., 2013; MONTEIRO et al., 2021; SWENSON et al., 2004). In six samples in the unstained sediment and in one sample stained with Gram, mixed populations originating from culture with growth of only bacilli-type bacteria were visualized. This result proves the effectiveness of staining the urinary sediment with Gram in the differentiation of cellular debris (SWENSON et al., 2011).

Our data suggest that the collection method does not interfere with the observation of false positive results, probably due to the previously established bacteriuria criteria for bacterial culture for the different methods of obtaining urine (SMEE et al., 2013). The false-positive results were due to the presence of small particles that resembled bacteria in size, shape and Brownian motion. These particles are usually small lipid molecules, cytoplasmic organelles, amorphous crystals or debris that do not grow in culture, but contribute to false-positive results due to the analysis of unstained urinary sediment (SWENSON et al., 2004). SWENSON et al. (2004) descreveu o mesmo efeito quando o sedimento foi corado pela coloração de Wright Giemsa. A coloração de Gram tem como vantagem a possibilidade de diferenciação das bactérias entre Gram-positiva e negativa (WAY et al., 2013). SWENSON et al. (2004) described the same effect when the sediment was stained with the Wright Giemsa stain. Gram staining has the advantage of being able to differentiate between Gram-positive and Gram-negative bacteria (WAY et al., 2013).

The stained sediment was not able to detect bacteriuria in 4 samples collected by catheterization and in one collected by voided speciemens. This occurrence cannot be attributed to a low count of Colony Forming Units (CFUs), since these were similar to the samples classified as having bacteriuria in the sediment analysis. Therefore, perhaps there

could have been contamination of the culture medium or poor preparation of the sediment, as suggested by WAY et al. (2013).

The empirical use of antibiotics has been considered the main cause of the emergence of microorganisms resistant to multiple drugs. The World Health Organization (WHO) (2019) considers this the biggest problem to be faced in public health in the next century. In Veterinary Medicine, the treatment of probable UTI based on the results of bacteriuria analysis of urinary sediment is the major cause of the indiscriminate use of antimicrobials. Therefore, our preliminary results strongly suggest that stained urinary sediment analysis should be routinely implemented to mitigate the false detection of bacteriuria in urinalysis, thus reducing the misprescription of antibiotics.

CONCLUSION

The Gram staining technique of urinary sediment significantly increases the detection of bacteria in sediment analysis and decreases the occurrence of false positive results in urinalysis. Therefore, as it is an easy, fast and low-cost technique, it should be implemented in the laboratory routine.

Acknowledgment

Este estudo foi financiado pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Financial code 001, Brazil.

CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest.

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Table 1: Detection of bacteriuria in urine samples from dogs obtained by catheterization or spontaneous urination, analyzed at the Laboratory of Veterinary Clinical Analysis – HVU – UFSM.

	Catheterization (n=40)	Voided specimen (n=60)
Unstained sediment	37	57
Stained sediment	15	33
Culture	10	15

Source: Own authorship

Table 2: Occurrence of false positive or negative results for bacteriuria in urine samples from dogs collected by catheterization or spontaneous urination analyzed at the Laboratory of Veterinary Clinical Analysis – HVU – UFSM

	Voided specimen		Catheterization	
	False negative	False positive	False negative	False positive
Stained sediment	1	3	4	5
Unstained sediment	1	14	-	22

Source: Own authorship; (-): not observed

Table 3: Comparison of identification of bacterial morphology in culture and optical microscopy methods (stained and unstained sediment)

		Unstained		Stained	
Morfology	Culture	N° correct	N° uncorrect	N° correct	N° uncorrect
Coccus	7	5	2	6	1
Bacillus	10	1	9	5	5
Mix*	8	1	7	1	7
Total	25	7	18	12	13

Source: Own authorship; Mix: bacillus e coccus*

1 Table 4: Frequency of the distribution of the morphological classification obtained by

2 Culture and Unstained Urinary Sediment

3

Culture	Absence	Coccus	Bacillus	Mix Population	Total
Absence	5 (12,5%)	35 (87,5%)	-	-	40
Coccus	-	6 (85,7%)	-	1 (16,7%)	7
Bacillus	-	4 (40%)	-	6 (60%)	10
Mix Population	1 (12,5%)	5 (62,5%)	-	2 (25%)	8

4 Source: Own autorship; (-): not observed

Table 5: Frequency of distribution of morphological classification obtained in Culture and Unstained urinary sediment

Culture	Absence	Coccus	Bacillus	Mix Population	Total
Absence	32 (80%)	8 (20%)	-	-	40
Coccus	1 (14,3%)	6 (85,7%)	-	-	7
Bacillus	2 (20%)	1 (10%)	6 (50%)	1 (10%)	10
Mix population	2 (25%)	4 (50%)	1 (12,5%)	1 (12,5%)	8

Source: Own authorship; (-) not observed

CONCLUSÃO

A partir da análise de dados pode-se que concluir que a realização do sedimento corado com Gram diminui, substancialmente o número de resultados falsos positivos, quando comparado a análise do sedimento não corado. Essa diminuição na falsa detecção de microorganismos está relacionada a maior capacidade da coloração de Gram de diferenciar estruturas parecidas com bactérias, tanto em forma como em tamanho.

Em adição, a análise do sedimento corado possibilitou que menos pacientes utilizassem antibioticoterapia de forma desnecessária, já que a observação do sedimento não corado, comumente está relacionada com resultados não acurados. A crescente prescrição de antibióticos de forma desnecessária favorece a resistência bacteriana, sendo um grave problema em Medicina Humana e Veterinária e tornando o tratamento de indivíduos, que realmente precisam desses medicamentos, cada vez mais difícil e oneroso.

O presente estudo relatou menores valores de sensibilidade, especificidade, valor preditivo negativo e positivo, menores que outros estudos. Essa diferença ocorreu, provavelmente, devido a diferença no tamanho da população e dos métodos de colheita de urina obtidos no nosso estudo. Portanto, novas pesquisas com maior número de amostras são necessárias para melhorar a eficiência da coloração de Gram na diferenciação de bactérias e debris celulares.

Dessa forma, nosso estudo auxiliará no direcionamento terapêutico mais eficiente dos pacientes, principalmente dos pacientes graves que não possuem condições de esperar os resultados da cultura bacteriológica.

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